

In re: Fischer et al.
Serial No. 10/019,087
Filed: December 19, 2001
Page 3

Amendment to the Claims

The following list of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

1. (Currently Amended) A method of diagnosing hemostatic dysfunction comprising an inflammatory condition, said method comprising:
 - a) adding ~~one or more reagents~~ at least one reagent comprising a divalent metal ion to a test sample from a patient comprising at least part of a blood sample from the patient in order to cause formation of a complex comprising at least C-Reactive protein and at least one human lipoprotein, while causing substantially no fibrin polymerization, ~~wherein the one or more reagents comprises a divalent metal ion;~~
 - b) measuring the formation of said complex over time ~~so as to derive a~~ time-dependent measurement profile;
 - c) determining a slope and/or total change in the time-dependent measurement profile so as to diagnose hemostatic dysfunction in the patient; and
 - d) correlating the increase in steepness of the slope with an increase likelihood of mortality associated with hemostatic dysfunction in the patient,
wherein the inflammatory condition is selected from the group consisting of an infection, sepsis, systemic inflammatory response syndrome (SIRS) and combinations thereof.
2. (Canceled).
3. (Currently Amended) The method according to claim 1, wherein ~~said metal ion is a~~ the divalent metal ion is selected from the group consisting of ~~one or more~~ of calcium, magnesium, manganese, iron ~~or~~ and barium.
4. (Previously Presented) The method according to claim 3, wherein said divalent metal ion is calcium.

In re: Fischer et al.
Serial No. 10/019,087
Filed: December 19, 2001
Page 4

5. (Previously Presented) The method according to claim 1, wherein said reagent comprises calcium chloride.
6. (Currently Amended) The method according to claim 1, wherein a clot inhibitor is ~~provided as part of said reagent or as part of an additional reagent~~ added to said test sample.
7. (Original) The method according to claim 6, wherein said clot inhibitor comprises one or more of hirudin, heparin, PPACK, I2581, and antithrombin.
8. (Original) The method according to claim 1, wherein the formation of said complex is correlated to the increase probability of death of the patient.
9. (Original) The method according to claim 8, wherein the greater the formation of said complex, the greater the likelihood of death of the patient.
10. (Original) The method according to claim 1, wherein the time dependent measurement profile is an optical transmission profile, and wherein the greater the decrease of optical transmittance through the test sample, the greater the formation of said complex, and the greater the likelihood of mortality of the patient.
11. (Currently Amended) The method according to claim 1, wherein said at least one human lipoprotein is selected from the group consisting of ~~comprises one or more of~~ chylomicrons or remnants thereof, VLDL, IDL, LDL, or HDL and combinations thereof.
12. (Previously Presented) The method according to claim 11, wherein diagnosing hemostatic dysfunction in the patient comprises a prediction of the likelihood of mortality of the patient.

In re: Fischer et al.
Serial No. 10/019,087
Filed: December 19, 2001
Page 5

13. (Currently Amended) The method according to claim 1, wherein said ~~one or more reagents~~ at least one reagent is added to said test sample in the absence of clot inducing reagents.

14. (Previously Presented) The method according to claim 1, wherein the formation of a precipitate is measured at least once after time zero.

15. (Previously Presented) The method according to claim 14, wherein a single endpoint measurement is made of precipitate formation after time zero.

16. (Currently Amended) The method according to claim 1, wherein said ~~one or more reagents~~ at least one reagent is capable of causing precipitate formation completely in the absence of fibrin polymerization.

17. (Currently Amended) The method according to claim 10, wherein the amount of fibrin polymerization, ~~if any~~, causes no change in optical transmittance.

18. (Currently Amended) A method for predicting an increased likelihood of system failure or mortality of a patient, comprising:

- a) obtaining a blood sample from a patient;
- b) obtaining plasma or serum from said blood sample;
- c) adding ~~one or more reagents~~ at least one reagent comprising a divalent metal ion capable of inducing the formation of a protein complex comprising at least one lipoprotein and at least one acute phase protein ~~wherein the one or more reagents comprise a metal ion~~;
- d) taking one or more measurements of a parameter of the plasma or serum and correlating the measured parameter to complex formation ~~if present~~; and
- e) correlating the formation of the complex to an increased likelihood of system failure or mortality of the patient.

In re: Fischer et al.
Serial No. 10/019,087
Filed: December 19, 2001
Page 6

19. (Currently Amended) The method according to claim 18, wherein a plurality of measurements are made after addition of said ~~one or more reagents~~ at least one reagent in order to derive a time-dependent measurement profile.

20. (Currently Amended) The method according to claim 18, wherein a ~~single~~ said at least one reagent includes a single reagent that is used prior to taking said measurements.

21. (Original) The method according to claim 18, wherein said measurements are measurements of optical transmission or absorbance through said sample.

22. (Canceled).

23. (Currently Amended) The method according to claim 18, wherein said divalent metal ion comprises one or more is selected from the group consisting of calcium, magnesium, manganese, iron or and barium.

24. (Currently Amended) The method according to claim 18, wherein adding said at least one reagent further comprises adding a clot inhibitor is provided as part of said one or more reagents to said plasma or serum.

25. (Currently Amended) The method according to claim 24, wherein said clot inhibitor ~~comprises one or more~~ is selected from the group consisting of hirudin, heparin, PPACK, I2581, or antithrombin and combinations thereof.

26. (Original) The method according to claim 18, wherein said one or more measurements are unaffected by clot formation due to lack of fibrin polymerization.

In re: Fischer et al.
Serial No. 10/019,087
Filed: December 19, 2001
Page 7

27. (Currently Amended) The method according to claim 18, wherein the taking one or more measurements comprises taking are a plurality of measurements, and wherein taking the plurality of measurements further comprises detecting a rate of change of said plurality of measurements and/or a total change of said plurality of measurements to determine the increased likelihood of system failure or mortality.

28. (Original) The method according to claim 18, wherein said at least one lipoprotein comprises VLDL, IDL and/or LDL, and said at least one acute phase protein comprises SAA and/or CRP.

29. (Original) The method according to claim 28, wherein a majority of said complex comprises CRP bound to VLDL.

30. (Original) The method according to claim 18, wherein the prediction of the increased likelihood of system failure or mortality is more accurate than in the absence of steps a) to e).

31. (Original) The method according to claim 18, wherein steps a) to e) are performed at least once more at a later time in order to determine patient condition regression or progression.

32. (Currently Amended) A method for diagnosis or monitoring of a hemostatic dysfunction comprising an inflammatory condition, said method comprising a confirmatory assay of determining the extent of inhibition of precipitation by a precipitate inhibiting reagent comprising:

- a) adding ~~one or more reagents~~ at least one reagent comprising a divalent metal ion to a test sample comprising at least a component of a blood sample from a patient in order to cause formation of a precipitate comprising an acute phase protein and a lipoprotein, ~~wherein the one or more reagents comprises a metal ion;~~

In re: Fischer et al.
Serial No. 10/019,087
Filed: December 19, 2001
Page 8

- b) measuring the precipitate comprising the acute phase protein and the lipoprotein;
- c) adding a precipitate inhibiting reagent, before or after adding said ~~one or more~~ precipitate causing reagents at least one reagent, which inhibits at least in part the formation of the precipitate; and
- d) determining the extent of inhibition of precipitation by said precipitate inhibiting reagent,

wherein the inflammatory condition is selected from the group consisting of an infection, sepsis, systemic inflammatory response syndrome (SIRS) and combinations thereof.

33. (Currently Amended) The method of claim 32, wherein said precipitate inhibiting reagent is added after all or substantially all of the lipoprotein has become associated with acute phase protein ~~so as~~ to form said precipitate.

34. (Original) The method of claim 32, wherein said precipitate inhibiting reagent is added prior to adding the precipitate causing reagent.

35. (Currently Amended) The method of claim 32, wherein said ~~precipitate causing reagent is a~~ divalent metal cation ion is selected from the group consisting of calcium, magnesium, manganese, iron, barium and combinations thereof.

36. (Canceled).

37. (Original) The method of claim 36, wherein said precipitate inhibiting reagent is capable of inhibiting the association of CRP with chylomicrons or remnants thereof, LDL, VLDL and/or IDL.

38. (Currently Amended) The method of claim 37, wherein the determining of the extent of inhibition is performed over time ~~so as~~ to derive a time-dependent measurement profile.

In re: Fischer et al.
Serial No. 10/019,087
Filed: December 19, 2001
Page 9

39. (Original) The method of claim 38, wherein the measurement over time is a measurement of optical transmittance or absorbance over time.

40. (Currently Amended) A method for diagnosis or monitoring of a hemostatic dysfunction comprising an inflammatory condition, said method comprising correlating the formation of a complex to a concentration of one or more lipoproteins comprising:

- a) providing a test sample from a test subject;
- b) adding ~~one or more reagents~~ at least one reagent comprising a divalent metal ion and at least one acute phase protein to said test sample in order to cause formation of a complex of one or more lipoproteins and one or more acute phase proteins, ~~wherein said reagent comprises a divalent metal cation and an acute phase protein;~~
- c) measuring the formation of the complex; and
- d) correlating the formation of the complex to a concentration of said one or more lipoproteins observed in patients with said hemostatic dysfunction, wherein the formation of an initial complex and the formation of an additional complex are measured over time ~~so as~~ to provide respective first and second time-dependent measurement profiles.

41. (Canceled).

42. (Previously Presented) The method of claim 40, wherein said acute phase protein is CRP.

43. (Previously Presented) The method of claim 40, wherein said one or more lipoproteins is chylomicrons, VLDL and/or IDL.

44. (Canceled).

In re: Fischer et al.
Serial No. 10/019,087
Filed: December 19, 2001
Page 10

45. (Currently Amended) The method of claim 40, further comprising correlating (i) wherein the measured additional complex and (ii) the measured initial complex together are correlated to a total amount of acute phase protein in the test sample.

46. (Currently Amended) The method of claim 40 ~~[[44]]~~, wherein the acute phase protein is C-reactive protein.

47. (Original) The method of claim 40, wherein the measured initial complex is correlated to a likelihood of system failure and/or mortality.

48. (Previously Presented) The method of claim 47, wherein the greater the initial complex measured, the greater the likelihood of system failure and/or mortality.

49. (Currently Amended) A method for testing the effectiveness of a therapeutic for treatment of hemostatic dysfunction, comprising:

- a) providing from a test subject a test sample to be tested for complex formation;
- b) adding one or more reagents which causes formation of a complex of acute phase protein and lipoprotein present in said test sample, wherein the reagent comprises a metal ion;
- c) administering to said test subject a therapeutic suspected of being useful in the treatment of hemostatic dysfunction;
- d) repeating steps a) and b); and
- e) determining if the amount of complex formed has changed,

wherein a decrease in the amount of complex formed correlates to the effectiveness of the therapeutic for treatment of hemostatic dysfunction.

50. (Currently Amended) A method of diagnosing hemostatic dysfunction comprising:

- a) adding ~~calcium~~ a ~~metal~~ divalent metal ion and one or more clot inhibitors to a blood sample from a patient in order to cause formation of a complex

In re: Fischer et al.
Serial No. 10/019,087
Filed: December 19, 2001
Page 11

comprising C reactive protein (CRP) and at least one human lipoprotein selected from the group consisting of very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL), while causing substantially no fibrin polymerization;

- b) measuring the formation of said complex over time so as to derive a time-dependent measurement profile; and
- c) determining a slope and/or total change in the time-dependent measurement profile; and
- d) correlating the formation of the precipitate to the likelihood of mortality, the greater the formation of said complex, the greater the likelihood of death of the patient.

51. (Previously Presented) The method of claim 50, wherein the hemostatic dysfunction is disseminated intravascular coagulation (DIC).

52. (Previously Presented) A method for testing the effectiveness of a therapeutic for treatment of hemostatic dysfunction, comprising (a) monitoring the formation of a complex comprising C reactive protein (CRP) and at least one human lipoprotein selected from the group consisting of very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL), and (b) correlating the decrease of complex formation with effectiveness of a therapeutic for treatment of hemostatic dysfunction.

53. (Previously Presented) The method of claim 52, wherein the hemostatic dysfunction is disseminated intravascular coagulation (DIC).

54. (Currently Amended) A method of diagnosing hemostatic dysfunction comprising an inflammatory condition, said method comprising:

- a) adding calcium to a blood sample from a patient in order to cause formation of a complex comprising at least C-Reactive protein and at least one human lipoprotein, while causing substantially no fibrin polymerization;

In re: Fischer et al.
Serial No. 10/019,087
Filed: December 19, 2001
Page 12

- b) measuring the formation of said complex over time so as to derive a time-dependent measurement profile; and
- c) determining a slope and/or total change in the time-dependent measurement profile; and
- d) correlating the formation of the precipitate to the likelihood of mortality, the greater the formation of said complex, the greater the likelihood of death of the patient.

55. (Currently Amended) The method of claim 50, wherein the metal divalent metal ion is calcium.